

Universidade de Lisboa  
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IN A LAKE SYSTEM OF CENTRAL AMAZON**

**ZULMIRA HELENA GAMITO COIMBRA DE ALMEIDA**

Dissertação de Mestrado em Biologia da Conservação

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## RESUMO

Devido à sua proximidade com as populações humanas, os golfinhos de rio estão entre os cetáceos mais ameaçados do mundo, e atualmente existem em apenas duas grandes regiões: as bacias dos rios Amazonas e Orinoco (com ocorrência de *Sotalia fluviatilis* na primeira e de *Inia geoffrensis* em ambas) e as bacias dos rios Indu, Ganges and Brahmaputra (com ocorrência de *Platanista gangetica*). Uma quarta espécie existiu no rio Yangtze (China) até 2006, ano em que foi considerada extinta.

As estimativas de abundância são essenciais para o estudo de populações ameaçadas, e a foto-identificação, juntamente com modelos de marcação-recaptura, têm sido usados com grande sucesso em estudos com cetáceos.

*S. fluviatilis* é a única espécie da família Delphinidae (família de golfinhos oceânicos) exclusiva de água doce, e o seu estatuto de conservação não está definido devido à falta de dados suficientes. Porque as populações da sua simpátrica *I. geoffrensis* têm sofrido grande desfalque nas últimas décadas devido à caça ilegal, maior atenção deveria ser prestada à tendência das populações de *S. fluviatilis*.

Dois estudos anteriores (realizados em 1980 e 2004) estimaram a densidade da espécie na região do Médio Solimões, e um outro estudo (1997) obteve valores de abundância para o Alto Amazonas.

O objectivo deste trabalho foi fazer a primeira estimativa de abundância de *S. fluviatilis* na região do Médio Solimões (Amazônia Central, Brasil), assim como elaborar um catálogo com os indivíduos identificados através da fotografia que possa servir como ponto de partida para um projeto de monitorização da espécie.

O estudo foi desenvolvido no Sistema de Lagos do Mamirauá, situado no Sul da Reserva de Desenvolvimento Sustentável Mamirauá, uma área protegida de floresta de várzea (com um ciclo hidrológico que faz o nível da água variar até cerca de 20 m em algumas áreas) a cerca de 500 km a Oeste da Cidade de Manaus. A área de estudo foi de cerca de 13,5 km<sup>2</sup>, dividida em três secções (lago e canal interno, canal externo e rio principal) e foi amostrada em 10 ocasiões ao longo de três meses da estação da cheia, durante as quais foram realizados transectos e fotografados todos os animais encontrados.

Um total de 104 grupos de *S. fluviatilis* (389 golfinhos) foram avistados e as fotografias de alta qualidade obtidas permitiram, com auxílio do programa DARWIN, identificar 97 animais com base num mínimo de três características individuais permanentes (dos quais 81 foram avistados em mais do que uma ocasião de amostragem). O número de indivíduos identificados

teve uma tendência para reduzir de uma ocasião de amostragem para a seguinte, oferecendo assim alguma confiança no esforço amostral empregue.

A secção de rio principal contou com 96,4% dos avistamentos, e nenhum golfinho foi avistado na secção do lago e canal interno. Sendo a secção de rio principal composta essencialmente por áreas de confluência de canais (conhecidas áreas de alimentação da espécie) e tendo o estudo sido realizado durante elevados níveis da água (durante os quais toda a terra existente na várzea fica submersa, possibilitando aos peixes refugiarem-se na floresta densa em vez de permanecerem nos lagos e canais onde ficam mais vulneráveis aos seus predadores), estes resultados seriam de esperar.

O programa CAPTURE foi utilizado para avaliar se a população poderia ser considerada fechada (ausência de nascimentos, mortes, imigração e emigração) e porque essa condição se verificou, o estimador de abundância zero-truncated Poisson-log normal (ZPNE), disponível no programa MARK, foi escolhido para determinar a abundância de *S. fluviatilis* em cada uma das ocasiões de amostragem, enquanto que uma análise de população (POPAN) disponível no mesmo programa estimou o tamanho de uma super-população hipotética da qual e para a qual os indivíduos podem sair e entrar.

O ZPNE permite a modulação de seis parâmetros (probabilidade de reavistamento, heterogeneidade individual, número de indivíduos não marcados, sobrevivência aparente, probabilidade de transição de um estado observável para um não observável e probabilidade de permanência num estado não observável), tendo todos eles sido modulados como constantes e variáveis ao longo do tempo, com exceção do terceiro que apenas foi modulado como variável (já que não havia razão para acreditar que a variação de abundância entre ocasiões de amostragem fosse exclusivamente devida à variação do número de indivíduos marcados), produzindo um total de 32 modelos, dos quais apenas dois foram considerados aceitáveis pelo princípio da máxima parsimónia ( $AIC_c < 2$ ). Os seus valores variaram entre 14 e 108 ( $IC\ 95\% = 10 - 142$ ), dependendo da ocasião de amostragem.

A POPAN permite a modulação de quatro parâmetros (probabilidade de sobrevivência, probabilidade de reavistamento, probabilidade de um indivíduo da super-população entrar na população amostrada e tamanho inicial da super-população), tendo os três primeiros sido modulados como constantes e variáveis ao longo do tempo e o quarto apenas como constante (já que apresenta um único valor possível de modular para todo o período de estudo), produzindo um total de oito modelos. Apenas um modelo obtido através da POPAN foi considerado aceitável ( $AIC_c < 2$ ), estimando uma super-população de 130 indivíduos ( $IC\ 95\% = 104 - 162$ ). Dado que os pressupostos das formulações ZPNE e POPAN foram cumpridos



(à exceção daquele que obriga a que todos os indivíduos tenham a mesma probabilidade de serem identificados quando utilizando o ZPNE, e cujo não cumprimento pode resultar numa subestimativa da abundância), os resultados foram considerados confiáveis. Os valores obtidos por ambas as formulações são complementares e indicam uma elevada importância da área de estudo para a super-população, já que em quatro das ocasiões amostrais pelo menos 70% da super-população estimada se encontrava nela presente. Porque é possível que os valores obtidos pelo ZPNE sejam inferiores aos reais (caso o sexto pressuposto não tenha sido cumprido), a abundância real de *S. fluviatilis* em cada ocasião de amostragem será ainda mais próxima daquela estimada pela POPAN. Esta aproximação de valores permite inferir que a estimativa para a abundância da super-população possivelmente representa a abundância da espécie na região estudada. A variação da abundância de *S. fluviatilis* entre ocasiões de amostragem pode ainda ter sido devida a factores não quantificados neste estudo, como por exemplo distribuição e movimentação de presas e velocidade da água.

Os resultados do presente estudo foram superiores em relação àqueles obtidos pelos três estudos anteriores acima referidos, o que provavelmente se deve a uma diferença entre as metodologias usadas, incluindo tamanho, tipo e localização geográfica das áreas estudadas.

Porque as marcas utilizadas na identificação dos 97 indivíduos são, na sua maioria, passíveis de serem reconhecidas mesmo após alargados períodos de tempo, sugere-se a utilização do catálogo fotográfico resultante deste estudo como ponto de partida para uma monitorização assídua da população de *S. fluviatilis* do Sistema de Lagos do Mamirauá.



## **ABUNDANCE OF *Sotalia fluviatilis* (DELPHINIDAE) IN A LAKE SYSTEM OF CENTRAL AMAZON**

### **ABSTRACT**

Because of their close contact with human populations, river dolphins are among the most menaced cetaceans worldwide. Today, only three strictly freshwater species remain, and *Sotalia fluviatilis*, endemic of the Amazon River basin, is the less known. The objective of this study is the estimation of the abundance of the species in a lake system of the Central Solimões River, Brazil. A total of 10 sampling sessions were conducted over three months. All sighted dolphins ( $n = 389$ ) were photographed, allowing the identification of 97 individuals. Two mark-recapture formulations for open populations available in program MARK were used. The zero-truncated Poisson-log normal estimator (ZPNE) estimated an abundance of 14 to 108 (95% CI = 10 - 142) dolphins in the study area, depending on the sampling occasion, while the population analysis (POPAN) estimated a super-population using that same area of 130 (95% CI = 104 - 162) animals. The complementary results obtained by these two formulations constitute the first estimate of *S. fluviatilis* abundance in Middle Solimões River. It is suggested that these values, along with the catalog of the 97 identified individuals, are used as a starting point to a project specially designed for monitoring *S. fluviatilis* in the region.

**Key words:** tucuxi; conservation; *várzea*; photo-identification; mark-recapture analyses.

## INTRODUCTION

River dolphins are among the least known and most endangered cetaceans of the world (Hamilton *et al.*, 2001). There are only two major regions where as few as three extant obligate freshwater species inhabit: (1) the Amazon and Orinoco River basins, in South America, with the presence *Sotalia fluviatilis* (Gervais, 1853) in the former and *Inia geoffrensis* (de Blainville, 1817) in both; and (2) the Indu, Ganges and Brahmaputra River basins, in Southern Asia, where *Platanista gangetica* (Lebeck, 1801) occurs (Hamilton *et al.*, 2001). Both *I. geoffrensis* and *S. fluviatilis*' conservation status are defined as data deficient and *P. gangetica* is considered endangered by the International Union for the Conservation of Nature (IUCN, 2013). Until 2006, a fourth species, *Lipotes vexillifer* (Miller, 1918), existed in the Yangtze River (China), but it was considered extinct after an intensive but vain attempt to sight any of the last 13 animals known to be alive in 2002 (Turvey *et al.*, 2007).

Habitat loss, accidental drowning in fishing gear, hunting, river damming, boat disturbance and pollution are the most invasive threats to river dolphins (da Silva, 2009; Flores and da Silva, 2009; Smith *et al.*, 2009; Zhou, 2009).

Abundance estimations are of great importance, for they are required to evaluate conservation status and population trends, as well as to help designing conservation measures. When working with dolphins, this is normally a challenging task because these animals are hard to capture, to handle, to mark and to locate. Photo-identification, mark-recapture and mark-resight (a conceptual variation of the previous) analyses have been used as successful non-invasive methods to estimate abundance of several cetaceans populations (*e.g.* Gormley *et al.*, 2005; Kaplan *et al.*, 2009; Ryan *et al.*, 2011; Félix *et al.*, 2011; Vernazzani, 2011; Baird *et al.*, 2013;).

The genus *Sotalia* was controversial for a long time, but after the genetic study by Cunha *et al.* (2005), what used to be a genus comprising a single species with riverine and marine ecotypes, is presently composed of two different species: *S. guianensis* (van Bénédén, 1864) (occurring in the East Central and South America coastal waters, from Honduras to the South of Brazil) and *S. fluviatilis* (inhabiting some areas of the Amazon River drainage, where it is commonly known as tucuxi) (Flores and da Silva, 2009).

Despite its uniqueness as the only member of family Delphinidae strictly living in freshwater habitats, little is known about *S. fluviatilis*. Although high entanglement risk has been reported for the species (Crespo *et al.*, 2010), bycatch trends are unknown (Iriarte and Marmontel, 2013). The sympatric *I. geoffrensis* (boto vermelho) populations have been suffering a drastic decrease due to active hunting with the purpose of using their meat as bait for the scavenger fish *Calophysus macropterus* (Lichtenstein, 1819) (da Silva *et al.*, 2011; Mintzer *et al.*, 2013), and although there is no evidence that this illegal activity poses a threat to *S. fluviatilis*, populations should be more attentively followed.

Projeto Boto, a long-term project in partnership with Instituto Nacional de Pesquisas da Amazônia, whose the main purpose is to monitor *I. geoffrensis* populations in the Middle Solimões River (da Silva and Martin, 2000), is the responsible for the majority of the literature about the tucuxi (*e.g.*, Best and da Silva, 1984; da Silva and Best, 1994, 1996; Martin *et al.*, 2004; Faustino and da Silva, 2006; da Silva *et al.*, 2007, 2010; Mello *et al.*, 2010). In the last two decades, 20 *S. fluviatilis* were captured by Projeto Boto. These animals were branded on both sides with freeze-branded characters and notched on the dorsal fin. They were then released, and several resightings of them have been recorded over the years (da Silva and Martin, 2000).

Only three studies were conducted in order to estimate *S. fluviatilis* numbers: Magnusson *et al.* (1980) estimated an index of density of 0.59 individuals per surveyed km after surveying Solimões River between Manaus and Jutica (550 km by river), Martin *et al.* (2004) estimated a density of 3.2 inds. km<sup>-2</sup> within a 50 km radius of the junction of Amazon and Japurá Rivers, and Vidal *et al.* (1997) estimated an abundance of 409 animals in a study area of 250 km<sup>2</sup> in the upper Amazon River.

The aim of this study was to make the first estimates of *S. fluviatilis* abundance in Middle Solimões River region, as well as to provide a starting point for monitoring the population trend through time, using photo-identification and recent analytical methods.

## **MATERIALS AND METHODS**

### **STUDY AREA**

The Mamirauá Sustainable Development Reserve (MSDR), is located about 500 km west of Manaus (Central Amazon, Brazil), comprises the area between Solimões and

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Japurá Rivers and is limited in the North by the Anarapu channel (Fig. 1a). With a total area of 11 000 km<sup>2</sup> it is the largest protected area of the Amazonian flooded forests in Brazil (Ayres, 1995).

The climate in MSDR is classified as *Af* (tropical rain forest) following Köppen-Geiger (Peel *et al.*, 2007), with mean annual precipitation of about 2 300 mm and mean seasonal temperatures between 22°C (rainy season) and 32°C (dry season) (SCM, 1996). The region is located in a vast plain originated by deposition of Andean sediments, about 50 m above sea level. It is affected by cyclic hydrologic variations that can range up to 15 – 20 m in some areas. The highest water levels normally occur in May and June, flooding all the land between Solimões and Japurá Rivers and creating the so-called *várzea* forests, while the lowest water levels occur between September and November. A variety of formations such as lakes, channels, islands, *restingas* and *chavascais* are abundant, creating a morphologically complex ecosystem (Ayres, 1995).

The study area is located within the Mamirauá Lake System (MLS), in the south part of the MSDR. It is composed of three sections, with a length of approximately 15 km each: (1) section X – the junction of Japurá and Solimões River (with an average width of about 1500 m in the region); (2) section Y – the outer part of the channel leading to Mamirauá Lake (with an average width of about 200 m), and (3) section Z – Mamirauá Lake and the inner part of the same channel (with an average width of about 300 m) (Fig. 1b).

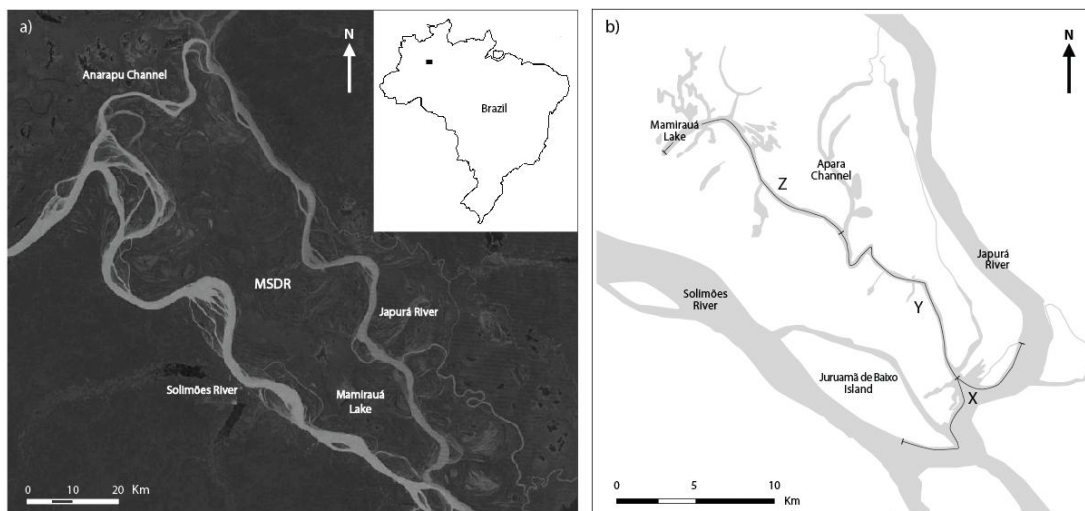


Figure 1 – Location of the Mamirauá Sustainable Development Reserve in Brazil (a) and Mamirauá Lake System in the Mamirauá Sustainable Development Reserve showing sections X, Y and Z, where the present study was developed (b). Adapted from da Silva and Martin (2000).

## **SURVEY METHODS**

A total of 10 sampling occasions were conducted during the high water level season, distributed from March to June of 2013. Each sampling occasion lasted two consecutive days with good visibility conditions (no rain, fog or high turbulence level). Sections Y and Z were normally sampled in the first day and section X in the second. Some *S. fluviatilis* groups were also photographed during opportunistic observations.

A 4.4 m engine boat was used as survey platform for the only observer. Because of considerable loss of visibility beyond 100 m, transects were performed twice per sampling occasion, both times parallel to the vegetation line. In section X, the first transect was performed 100 m and the second 300 m away from vegetation line, sampling a total width of 400 m. In section Y and Z the first and the second transects were performed 75 m away from right and left margins, respectively, corresponding to the full width of these river sections.

A total of about 13.5 km<sup>2</sup> was sampled. Every time a group of *S. fluviatilis* (one or more tucuxis, swimming together within a maximum distance of 50 m among each other) was sighted, it was followed until good photographs of the dorsal fin of all present individuals were taken, using a digital camera equipped with a 70 – 210 mm lens. The minimum group size (either the maximum number of individuals sighted simultaneously jumping out of water, or the maximum number of individuals sighted not simultaneously jumping out of water but counted as different animals considering time, position and direction between sightings) was also recorded. Transects were then resumed from the point of interruption at the same constant speed.

In section X, the width of the transects was settled to be about 200 m to the left and 200 m to the right of the path followed, and did not comprise the whole river width because of loss of visibility beyond such distance. Because sections Y and Z were at most 400 m wide, transects were made along the center of the channels and all their width was considered.

## **PHOTO-IDENTIFICATION**

Among all the photographs taken, only those allowing detection of the most subtle individual marks in dorsal fins and allowing to evaluate if the individual was or was not

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marked were selected, whether they were taken perpendicular to the fins or not. Individual marks could be both natural or human-caused and included: a characteristic dorsal fin profile, scratches, scars, nicks, notches (natural or artificial), tears, lack of pigmentation and freeze-branded characters. Program DARWIN (Hale, 2008) was used to help comparing photographs and creating a database of identified individuals. A new animal was added to the database every time at least three characteristic marks of its dorsal fin didn't match those of any other. In order to know the number of unmarked tucuxis in each sampling occasion, good photographs of immaculate or very poorly marked individuals were used to distinguish unmarked animals within that period, even if subtle individual marks used were not sufficient for confident re-identification after a longer time. Such individuals were not included in the final catalog.

#### **ANALYTICAL METHODS**

Program CAPTURE (Otis *et al.*, 1978) was used to perform a closure test in order to evaluate if the assumption of closed population (no births, deaths, immigration or emigration) could be accepted within sampling occasions (48 hours). For each identified individual, a sighting history was built in the shape of a string of 10 characters, representing the 10 sampling occasions, where "0" means "not sighted" and "1" means "sighted". Opportunistic observations were not included in this analysis, because the program does not provide a way to inform the existence of an animal available for resighting but never resighted during sampling occasions.

Because CAPTURE's closure test might have failed due to its difficulty in distinguishing failure in closure from variation in capture probabilities (Otis *et al.*, 1978), ZPNE and POPAN open population models were used to estimate population size instead of the more traditional closed population models.

The zero-truncated Poisson-log normal estimator (ZPNE), a mark-resight model developed by McClintock *et al.* (2009) and McClintock and White (2009) and available in the program MARK (White and Burnham, 1999) was chosen among other mark-resight formulations for allowing geographic openness and sampling with replacement between primary sampling intervals, as well as for allowing unknowingness of the total number of marked animals. The main assumptions of this model are: (1) geographic and demographic closure during primary sampling intervals, (2) no loss of marking within primary sampling



intervals, (3) no errors in distinguish marked and unmarked animals, (4) equal resighting rates for marked and unmarked animals, (5) unmarked animals sampled with no replacement within primary sampling intervals and (6) all animals have some chance of being sampled.

Because a period of two days was a short enough time to accept the assumption of closed population, each sampling occasion was considered a primary interval. Primary intervals were not equally distributed during the whole study period due to unfavorable climatic conditions, and therefore time intervals were set accordingly to the number of days between consecutive sampling occasions.

For each identifiable individual in the database, a sighting history was built, indicating how many times it was sighted during each primary interval. This time, tucuxis identified exclusively in opportunistic observations were also included in the analysis, since ZPNE has the capacity to deal with individuals known to be available for detection but never encountered during transects. Additionally, information on unmarked and marked but unidentified individuals was also included in the abundance estimation process, which increased the accuracy of the estimation.

Six parameters are possible to model when using ZPNE: (1) mean resighting probability (on a log scale) during primary interval  $i$  -  $\alpha_i$ , (2) individual heterogeneity level (on a log scale) during primary interval  $i$  -  $\sigma_i$ , (3) number of unmarked individuals in the population during primary interval  $i$  -  $U_i$ , (4) apparent survival between primary intervals  $i$  and  $i + 1$  -  $\phi_i$ , (5) probability of transitioning from an observable state at time  $i$  (e.g. on the study area) to an unobservable state at time  $i + 1$  (e.g., off the study area) -  $\gamma_i$  and (6) probability of remaining at an unobservable state at time  $i + 1$  (e.g. off the study area) when at an unobservable state at time  $i$  -  $\gamma'_i$ . Derived parameter  $N$ -hat values vary depending on the used model and gives the population size for each primary interval.

Parameters were modeled as constant across sampling occasions (.) and as time dependent (t), except for  $U$ , which was modeled only as time dependent because there was no reason to think that the population size variation between sampling occasions was due only to variation of marked individuals. All possible combinations for this modeling were run, creating an initial set of 32 models. As suggested by Burnham and Anderson (2009), Akaike Information Criterion with a small sample size correction factor ( $AIC_c$ ) was used to compare these models with respect to most parsimonious fit to the data. Then, acceptable

models (with  $\Delta AIC_c < 2$ ) (Burnham and Anderson, 2009) had their individual heterogeneity parameter fixed to zero in order to evaluate if models where individual heterogeneity was inexistent better fitted the data.

POPAN (Schwarz and Arnason, 1996) is a formulation based on the original Jolly-Seber model (Jolly, 1965; Seber, 1965), available in program MARK and allows abundance estimation for open populations by considering the existence of a super-population to and from where animals can migrate. POPAN's assumptions are the following: (1) no loss of marking during the study, (2) no errors in distinguish marked and unmarked animals, (3) sampling is instantaneous, (4) survival probabilities are equal for all animals between each pair of sampling occasions (homogeneous survival) and (5) equal catchability for marked and unmarked animals (homogeneous catchability).

Four parameters are possible to model when using POPAN: (1) survival probability between sampling occasions  $i$  and  $i + 1 - \phi_i$ , (2) resighting probability at sampling occasion  $i - p_i$ , (3) probability that an individual from the super-population would enter the population between sampling occasions  $i$  and  $i + 1$  ( $pent_i$ ) and (4) initial super-population size  $- N$ . The derived parameter  $N$ -hat estimates the super-population size.

As suggested by Schwarz and Arnason (1996), link function *sin* was used for parameters  $\phi$  and  $p$ , while MLogit(1) and Log were used for parameters  $pent$  and  $N$ , respectively. Survival probability, resighting probability and entrance probability were both modeled as constant and time dependent, while super-population size was modeled as constant only (eventual birth and deaths were considered neglectable given the short study period). All possible combinations of this modeling resulted in a set of 8 models that were compared using  $AIC_c$ , as described previously.

## RESULTS

A total of 104 *S. fluviatilis* groups (389 dolphins) were sighted during the 10 sampling occasions. The number of sighted dolphins varied greatly in space, the most part occurring in the junction of the main channels. No sightings were recorded for section Z and only 14 sightings occurred in section Y, while section X counted with 375 (96.4%) of total sightings (Fig. 2).

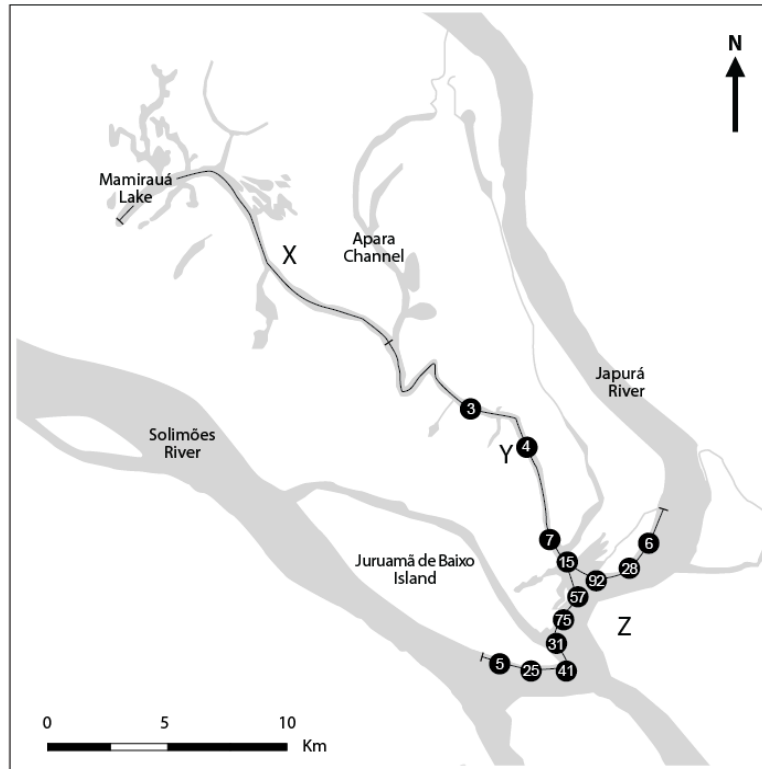


Figure 2 – Total minimum number of *S. fluviatilis* sighted per sighting spot during the 10 sampling occasions in the Mamirauá Lake System.

The interval between sampling occasions was not uniform due to atmospheric conditions. During the study, the water level raised from 31.34 m to 35.35 m above sea level (Instituto de Desenvolvimento Sustentável Mamirauá, 2013). The number of identified individuals varied over sampling occasions from 3 to 24, the number of newly identified individuals between 1 and 22, the number of unmarked individuals between 2 and 6 and the number of marked but unidentified individuals between 3 and 12 (Tab. 1).

About 20 000 photographs were analyzed, and 1 500 (7,5%) were considered useful to identify individuals, resulting in the production of a catalog where a total of 97 tucuxis were represented. Individual characteristic marks like dorsal fin shape, notches, tears and lack of pigmentation were considered stable enough during the three months study period, and therefore, the identification of the 97 individuals in the catalog, which was made based on such characteristics, was considered accurate.

Very slight differences between animals were used to distinguish them in the time period of 48 hours, but in dubious situations (*e.g.*, when absence of any detectable unique

characteristics occurred) different pictures were considered to be of the same animal, which might have led to underestimation of the number of unmarked individuals.

Table 1 – Sampling occasions, interval length between them, water level, number of identified, newly identified, unmarked and marked unidentified individuals per sampling occasion

Sampling occasions	Interval length (days)	Water level (m above sea level)	No. identified individuals	No. newly identified individuals	No. unmarked individuals	No. marked unidentified individuals
3-4/3/2013	-	31.34 - 31.39	22	22	2	8
9-10/3/2013	4	31.69 - 31.75	9	5	2	7
23-24/3/2013	12	32.98 - 33.08	21	15	3	3
30-31/3/2013	5	33.57 - 33.62	13	9	3	3
6-7/4/2013	5	33.92 - 33.97	11	2	2	7
19-20/4/2013	11	34.42 - 34.47	24	15	6	12
3-4/5/2013	12	34.92 - 34.95	21	7	5	7
18-19/5/2013	13	35.29 - 35.33	3	1	2	3
25-26/5/2013	5	35.34 - 35.35	13	4	2	3
1-2/6/2013	5	35.36 - 35.35	7	2	4	7

Of the 97 catalogued dolphins, 81 were identified during sampling occasions, and 16 during opportunistic observations only. Of the 81 individuals identified during transects, 38 ( $\approx 47\%$ ) of them were sighted in more than one sampling occasion (Fig. 3a). The number of newly identified individuals had a tendency to decrease from a previous sampling occasion to the next, as shown by the curve of cumulative number of identified individuals (Fig. 3b).

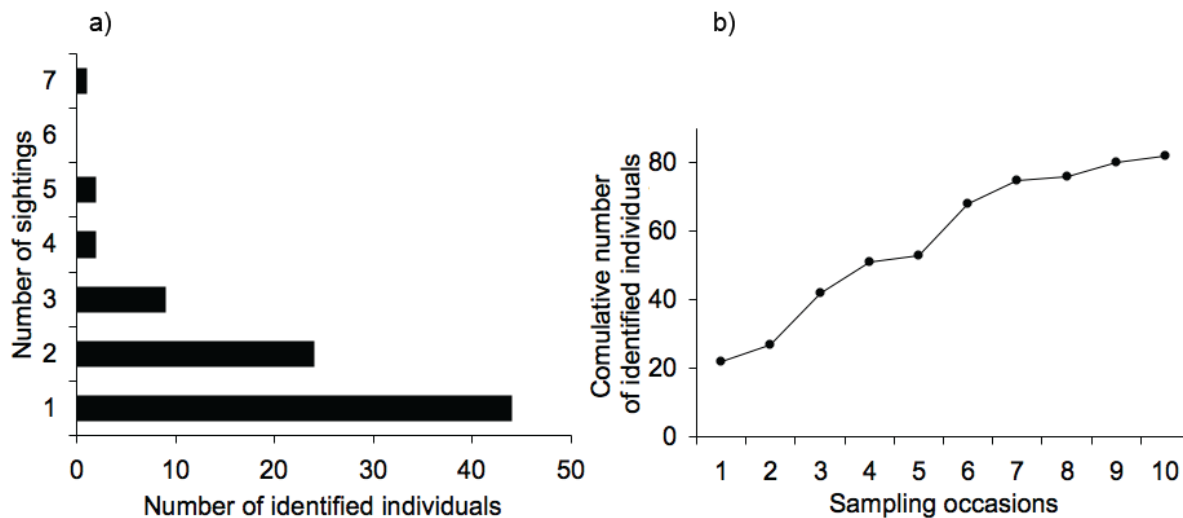


Figure 3 – Number of times identified dolphins were sighted during all sampling occasions (a), and cumulative number of identified individuals over sampling occasions (b).

The statistic value (z-value) for the closure test run in program CAPTURE was -0.320 and the significance level of the test was 0.374, which means that the assumption of closed population is probably acceptable, providing strong confidence in admitting closure within the period of time taken to perform a single sampling occasion (48 hours).

Three acceptable models were achieved using ZPNE. Among them, the one that was considered the most parsimonious was  $\{\alpha(.)\sigma(.)U(t)\phi(t)\gamma''(t)\gamma'(t)\}$ , with an  $AIC_c$  value of 524.4385. Models with individual heterogeneity fixed to zero were not among those with lowest  $AIC_c$  (Tab. 2). Acceptable models indicate that resighting probability ( $\alpha$ ) and individual heterogeneity ( $\sigma$ ) are constant through time, while the number of unmarked animals ( $U$ ) and the probability of remaining at an unobservable state ( $\gamma''$ ) are time dependent (Tab. 2). Summing the  $AIC_c$  weights for the two models with  $\phi(t)$  and after converting the proportion to percent, the apparent survival has approximately a 60% probability of being time dependent and a 40% probability of being constant. Proceeding in the same way, the probability of an individual to remain at an unobservable state ( $\gamma'$ ) has 76% probability of being time dependent and 24% probability of being constant.

Table 2 – List of models with lowest  $AIC_c$  values and respective characteristics achieved by the use of zero-truncated Poisson-log normal estimator in program MARK

Model	$AIC_c$	$\Delta AIC_c$	$AIC_c$ weight	Model likelihood	No. Parameters	Deviance
$\{\alpha(.)\sigma(.)U(t)\phi(t)\gamma''(t)\gamma'(t)\}$	524.4385	0.0000	0.39876	1.0000	18	483.3719
$\{\alpha(.)\sigma(.)U(t)\phi(.)\gamma''(t)\gamma'(t)\}$	524.6604	0.2219	0.35689	0.8950	18	483.5937
$\{\alpha(.)\sigma(.)U(t)\phi(t)\gamma''(t)\gamma'(. )\}$	525.8198	1.3813	0.19988	0.5012	18	484.7531
$\{\alpha(.)\sigma(.)U(t)\phi(.)\gamma''(t)\gamma'(. )\}$	528.8257	4.3872	0.04447	0.1115	17	490.3257

Considering the two models with lowest  $AIC_c$  values and highest  $AIC_c$  weights ( $\{\alpha(.)\sigma(.)U(t)\phi(t)\gamma''(t)\gamma'(t)\}$  and  $\{\alpha(.)\sigma(.)U(t)\phi(.)\gamma''(t)\gamma'(t)\}$ ), the population size estimation along the 10 sampling occasions varied between 14 and 108 individuals, and the lowest and the highest 95% confidence intervals are 10 and 142 individuals, respectively (Fig. 4).

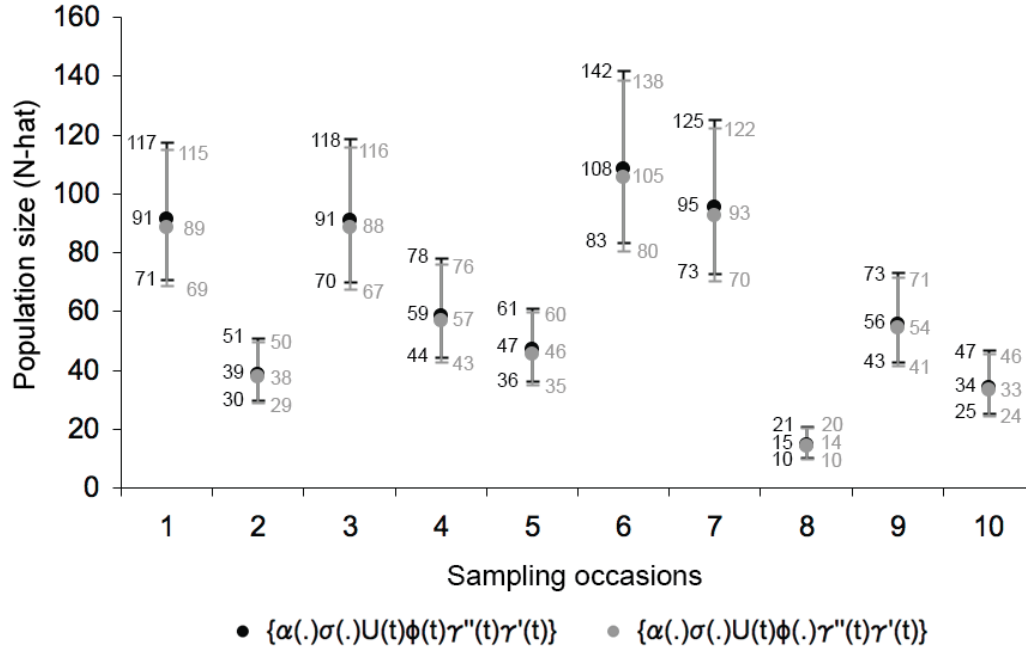


Figure 4 – Population size values given by models  $\{\alpha(.)\sigma(.)U(t)\phi(t)\gamma''(t)\gamma'(t)\}$  and  $\{\alpha(.)\sigma(.)U(t)\phi(.)\gamma''(t)\gamma'(t)\}$  and respective 95% confidence intervals.

Based on the result of the closure test, it is possible to assume that the population is closed within each primary interval (48 hours), respecting the first assumption of the ZPNE model. If some births, deaths, immigration or emigration occurred within this interval, it might have been in very small numbers and therefore negligible. The second assumption was also fulfilled, since loss of reliable marks for identification could not have occurred during the study period. Because only high quality pictures allowing detection of very slight marks were used in the photo-identification process, there is a good level of confidence in assuring that no errors in distinguishing marked and unmarked animals occurred, and that the third assumption was met. Because the marks are not detectable at a distance during the sightings, but only afterwards through visualization of the digital photographs in a computer screen, the observer's attention was randomly distributed among all group members, respecting the fourth assumption. As explained before, slight differences between individuals were used to distinguish dolphins within sampling occasions. Because unmarked individuals were not summed to the number of unmarked animals for a sampling occasion when identification doubts existed, the fulfillment of the fifth assumption was assured. The sixth assumption is potentially the one that might have been violated, because some animals could indeed have been less susceptible of being

photographed (*e.g.* for being more shy or cautious) than others. If that was the case, the estimation of the population abundance has excluded such individuals. Because none of the models with individual heterogeneity fixed to zero were among those with  $AIC_c < 2$ , and because this parameter is constant in models with such  $AIC_c$  values, there could indeed have occurred the violation of the sixth assumption.

Among models achieved using POPAN, only model  $\{\phi(.)p(t)pent(.)N(.)\}$  was considered acceptable, supporting the hypothesis that the survival probability ( $\phi$ ) and entrance probability ( $pent$ ) are constant through time, and that resighting probability ( $p$ ) is time dependent (Table 3). Model  $\{\phi(.)p(t)pent(.)N(.)\}$  estimated a super-population size of 130 individuals, with 95% lower and upper confidence intervals of 104 and 162, respectively.

Table 3 – List of models with lowest  $AIC_c$  values and respective characteristics achieved by the use of POPAN in program MARK

Model	$AIC_c$	$\Delta AIC_c$	$AIC_c$ weight	Model likelihood	No. Parameters	Deviance
$\{\phi(.)p(t)pent(.)N(.)\}$	422.0440	0.0000	0.88436	1.0000	13	0.0000
$\{\phi(.)p(t)pent(t)N(.)\}$	427.0737	5.0297	0.07152	0.0809	17	0.0000
$\{\phi(.)p(.)pent(t)N(.)\}$	428.3814	6.3374	0.03719	0.0421	6	0.0000
$\{\phi(t)p(.)pent(t)N(.)\}$	431.7572	9.7132	0.00688	0.0078	14	0.0000
$\{\phi(t)p(t)pent(t)N(.)\}$	441.6711	19.6271	0.00005	0.0001	25	0.0000

As covered previously relatively to correspondent parameters of ZPNE, first, second and fifth POPAN assumptions were not violated. The third assumption was also accomplished since the resighting technique does not involve handling the animals. Because dolphins are long lived and the study period was short enough to neglect eventual (although unlikely) deaths, we consider that survival probabilities are equal for all animals between each pair of sampling occasions, respecting the fourth assumption.

## DISCUSSION

The present study was carried out during very high water levels (from 31.34 m to 35.35 m), when all the land around the MLS was already flooded. Knowing that *S. fluviatilis* does not enter the flooded forest (Martin *et al.*, 2004; da Silva *et al.*, 2010), while their prey do in order to feed and avoid predation (Goulding, 1980), it is expectable that very

low numbers of tucuxis are found in the inner channels in this time of the year, as revealed in the results obtained (Fig. 2).

The number of sampling occasions was considered enough to identify marked individuals in the studied population, since the curve of cumulative number of identified individuals had a noticeable tendency to stabilize (Fig. 2b). The percentage of individuals sighted in more than one sampling occasion (47%) also gives some confidence regarding the adequacy of sampling effort (Fig. 2a).

As stated above, the assumptions for the used analytical methods were considered basically fulfilled, proving the adequacy of the chosen methodology to address the problem of the abundance estimation of *S. fluviatilis*, as well as providing a high degree of confidence in the results. However, some care in using the results should be taken, since it is possible that some individual heterogeneity exists, making some animals less susceptible of being captured by the camera than others, which might have resulted in the underestimation of the abundance.

The low abundance obtained on the eighth sampling occasion was due to a sudden change of visibility conditions, when water surface became choppy during transects in section X. The variation of *S. fluviatilis* abundance estimated by ZPNE on the other nine sampling occasions (33 to 108 individuals) can be explained by several factors. Because the majority of the sighted animals occurred in main channels junctions, known to be important feeding areas (Martin *et al.*, 2004), factors like variation in fish movement, distribution and abundance might also have determined *S. fluviatilis* abundance variance between sampling occasions. Eventual variation in water velocity over the study period might also have influenced the estimation of the tucuxis abundance, since the species is known to avoid very high water velocity areas (Martin *et al.*, 2004).

The abundance information obtained through the formulations ZPNE (14 to 108 individuals) and POPAN (130 individuals) are complementary, since the former provides an estimation for the number of individuals present in the study area during each sampling occasion (which was variable), while the second estimates the size of the super-population that uses the study area, to and from where dolphins can migrate (which is constant, since the shortness of the study period allows neglecting eventual births and deaths). Although the values obtained through the two formulations are numerically different, they are biologically compatible. During sampling occasions one, three, six and seven, at least 70%



of the 130 individuals composing the super-population were estimated to be present in the study area. ZPNE values might have been underestimated, since uncertainty exists about the fulfillment of its sixth assumption, which means that the real abundance in each sampling occasion is actually closer to the values obtained by POPAN. Because of this very likely coincidence of values, it is acceptable to admit that POPAN estimation represents the actual numbers of *S. fluviatilis* in the region, supporting the result of CAPTURE's closure test. This idea is also compatible with the fact that individuals move in home ranges larger than the study area, as known to be true by resighting records of some individuals marked by Projeto Boto in and outside present study's study area (unpublished data). Future studies using telemetry should be conducted in order to clarify in what patterns, if any, tucuxis actually move in space, as well as to help determine species' home range area.

Because present study's objective was to estimate the abundance of *S. fluviatilis* in Central Amazon, and because previous studies aiming to estimate specie's numbers in the region focused on density (Magnusson et al., 1980; Martin *et al.*, 2004), no direct comparison can be made. However, if an average of the abundance values obtained by ZPNE model  $\{\alpha(.)\sigma(.)U(t)\phi(t)\gamma''(t)\gamma'(t)\}$  (69 dolphins, obtained by averaging abundance values for all sampling occasions but the eighth, which was excluded for being an outlier) is considered, and knowing that the sampled area is about 13.5 km<sup>2</sup>, the index of density will be 5.1 *S. fluviatilis* km<sup>-2</sup>. This value is considerably higher than those achieved by Magnusson *et al.* (1980) (0.56 inds. km<sup>-1</sup>) and Martin *et al.* (2004) (3.2 inds. km<sup>-2</sup>). The difference verified may be due to a variety of reasons, starting by the different methods used. The main channel of Solimões River surveyed by Magnusson *et al.* (1980) greatly differs from the habitat type sampled in the present study, mainly composed by narrower channels and channel junctions. The fact that the latter are main feeding areas might have contributed to higher abundance values, added to the fact that the area covered in the present study is much smaller than the one surveyed in 1980. Also, because of the cyclical hydrologic variation, *S. fluviatilis* distribution varies greatly over the year, occurring in narrow channels and inner lakes during the high water level season, but evading from them when the water level decreases to avoid being trapped (Faustino and da Silva, 2006). This produces a variation of *S. fluviatilis* density in the Amazonian *várzea* along the year, since during very high and very low water levels dolphins will be more concentrated in main

channels. (Martin *et al.*, 2004). For this reason, density values might also fluctuate if only main channels are surveyed, explaining the difference between values obtained by Martin *et al.* (2004) and the present study.

Vidal *et al.* (1997) calculated an abundance of 409 *S. fluviatilis* in a study area of 250 km<sup>2</sup>. Because the span of the super-population hypothesized by POPAN is unknown, the index of density was again used to compare both studies. Again, the present study obtained a more elevated density value (5.1 inds. km<sup>-2</sup>) when compared with 1.6 inds. km<sup>-2</sup> obtained by Vidal *et al.* (1997). This can be explained by the fact that the study area of the previous study is located in the west extreme of species natural distribution (Flores and da Silva, 2009).

As experienced by Projeto Boto's observers, although freeze-branded characters normally fade away after some 5 or 6 years, notches made by researchers during capturing events allow recognition of individuals for at least 15 years (unpublished data). Some natural marks are as durable as such notches and therefore also useful to identify individuals even in large time periods. Because dolphins with durable marks are present in the catalog, we suggest using it as a starting point for a regular project specifically designed to monitor *S. fluviatilis* populations in MLS using photo-identification based on both natural and artificial marks.

The fact that sympatric *I. geoffrensis* has been suffering huge human pressure over the last two decades, and considering that the two species are ecologically similar, an alert should be considered to the need of a careful determination of *S. fluviatilis* population trends.

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